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(54) A differential labelling method using platinum complexes

(57) The invention relates to a method for differentially labelling one or more entities, together comprising distinct sulphur and nitrogen containing reactive sites. The invention further relates to an entity that has been labelled by a method according to the invention and to a diagnostic kit comprising a labelled entity and to a diagnostic kit to employ a method according to the invention.

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Description

[0001] The invention relates to a method for differentially labelling one or more entities, together comprising distinct reactive sites, to an entity that has been labelled by a method according to the invention and to a diagnostic kit for employing a method according to the invention.

[0002] An entity may be labelled with a detectable marker to detect, visualise, quantify or monitor the entity *e.g.* in chemical, biological or medical research or diagnosis. A wide variety of labelling methods are known from the art (for a review see Hermanson, 1996, Bioconjugate techniques, Academic Press, ISBN 0-12-342335-X).

[0003] Many factors may play a role in choosing a particular detectable marker and a particular method of labelling. Such factors include the nature of the entity, reaction conditions, detection limits of the labelled entity, sensitivity during the labelling reaction and specificity towards the entity.

[0004] Methods using platinum compounds to label bio-organic molecules have been considered interesting for a very long time. Various types of detectable marker moieties can be adhered to ionic platinum. Platinum compounds may react with a variety of reactive moieties on an entity.

[0005] The use of a *cis*-platinum compound has been described in European patent application no. 95201197.1. Herein a method is disclosed for linking bio-organic molecules and markers through *cis*-platinum compounds, of which two co-ordination sites are occupied by two ends of a stabilising bridge, such as an ethylene diamine group. These known *cis*-platinum compounds are suitable for linking labels to several kinds of bio-organic molecules, such as peptides, polypeptides, proteins, and nucleic acids. Methods using *trans*-platinum compounds have also been reported (EP application 97201066.4) to be suitable to label a variety of bio-organic molecules.

[0006] The reactivity of platinum compounds towards a variety of reactive sites is a benefit in many applications, since it may allow fast labelling reactions and an excellent sensitivity towards a wide variety of entities.

[0007] It may however be desired to direct the label to a specific reactive site of an entity, *e.g.* to improve the selectivity of the labelling. Also, pre-selected sites may be labelled in complex samples such as those samples comprising various types of bio-organic compounds. Differential or selective labelling often circumvents the need of sample purification and may be directed in such a way that targeted entities do not loose their native characteristics, *e.g.* 3D structure, activity, avidity, *etc.*

[0008] Furthermore it may be advantageous to label an entity at a controlled number of reactive sites. This may improve accuracy of the quantification and facilitate identification of a labelled entity. Such an improvement would be very valuable for various applications such as in the organochemical, biological or medical fields.

[0009] Moreover it is often a challenge in labelling chemistry to find a labelling method that does not affect the structure or the activity of an entity, *e.g.* of an enzyme, an immunoglobulin or a DNA-probe, to a high extent.

[0010] It is an objective of the present invention to provide a method to differentially label one or more entities together comprising distinct reactive sites, at a targeted reactive site.

[0011] Surprisingly it has been found that according to the invention one or more entities can be labelled through a platinum-linker. In a preferred embodiment said linker is a platinum-linker, and said entities together comprise one or more sulphur containing reactive sites and/or one or more nitrogen containing reactive sites, wherein a complex of a platinum compound and a marker is formed, and wherein said platinum compound is reacted with said one or more entities. In a preferred embodiment of the invention substantially only sulphur containing reactive sites or substantially only nitrogen containing reactive sites are linked to said platinum compound.

[0012] Entity as used herein is to be interpreted as something that comprises one or more sulphur containing reactive sites and/or one or more nitrogen containing reactive sites. In particular an entity relates to an inorganic or organic compound, including a bio-organic compound. A bio-organic compound as used herein refers to a biological carbon containing compound. Also, a bio-organic compound refers to a compound capable of inducing or affecting an action in a biological system, *e.g.* by inducing or affecting a therapeutic or prophylactic effect, an immune response, a metabolic process *etc.* "Entity" further relates to a micro-organism, a virus or a prion, or to a material comprising one or more of said sulphur reactive or nitrogen reactive types of reactive sites, or a product made thereof, such as a micro-array, a microtitre plate, a test strip or a test tube. Distinct reactive sites -which are to be labelled differentially - may be present together in one entity or in a combination (a mixture, a solution, a dispersion *etc.)* of more entities having only one or some of the to be labelled reactive sites, but together comprising said distinct reactive sites. Such a combination is for example a combination of an entity with only a nitrogen containing reactive site and an entity with only a sulphur containing reactive site.

[0013] In principle, any type of nitrogen containing reactive site or sulphur containing reactive site may be labelled using a method according to the invention. Preferred reactive sites include reactive sites comprising a primary amine, a secondary amine, a tertiary amine, an aromatic amine, a thiol, a thioether, a sulfide, a disulfide, a thioamide, a thion, an amide, an imide, an imine, an iminoether,or an azide. Examples of entities that can be labelled are entities chosen from the group of amino acids (preferably methionine, cysteine, histidine, lysine, and tryptophan), peptides, oligopeptides, polypeptides, proteins, immunoglobulins, enzymes, synzymes, phospholipides, glycoproteins, nucleic acids, nu-

cleosides, nucleotides, oligonucleotides, polynucleotides, peptide nucleic acids, peptide nucleic acid oligomers, peptide nucleic acid polymers, amines, aminoglycosides, nucleopeptides, and glycopeptides. Preferably in accordance with the invention, the entity is chosen from the group of amino acids, peptides, oligopeptides and polypeptides.

[0014] An entity linked to a platinum compound may be referred to as a Pt-S adduct (when attached to a sulphur containing reactive site), to a Pt-N adduct (when attached to a nitrogen containing reactive site), or in general to a Pt-adduct.

[0015] A sulphur containing reactive site may hereafter be referred to as a S-reactive site, and a nitrogen containing reactive site may hereafter be referred to as N-reactive site.

[0016] With a platinum linker, a platinum moiety is meant that can be used to couple a marker to an entity. A preferred linker compound as used in this invention is a platinum compound to which ligands are bound.

[0017] It has been found that a method according to the invention is highly suitable to direct the labelling of an entity towards a specified reactive site within an entity or a group of entities that together comprise a variety of reactive sites. Furthermore a method according to the invention has been found to have an excellent sensitivity towards the targeted (reactive site of the) entity, even in complex matrices. The prowess of a method according to the invention to distinguish to which reactive site a marker is labelled is *inter alia* extremely beneficial for analytical purposes. Not only may the excellent selectivity contribute to the accuracy and the dynamic range of quantification, but it also may improve the homogeneity of the labelled entity. The improved homogeneity generally has a beneficial effect upon band broadening during separation of a sample for analysis or for purification, *e.g.* by a chromatographic or electrophoretic method.

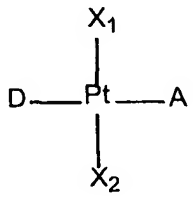
[0018] Furthermore it has been found possible to selectively label an entity without significantly affecting the structure or activity of a labelled entity, even if such an entity contains a fragile or labile part. This is a highly advantageous feature of the invention since it facilitates the detection or monitoring of a labelled entity while the entity retains activity - preferably substantially all of its activity - *in vivo* or *in vitro*. To the benefit of retaining activity, it has been found possible to direct labelling of an entity, like an immunoglobulin, an enzyme, a hormone, a nucleic acid in such a way that essentially no marker is labelled at one or more N- or S-reactive sites at a functional part of said entity.

[0019] Furthermore it was found that the present invention can be used to label an entity in such a way that the configuration of the entity largely remains unaffected after the entity has been labelled. This embodiment of the invention is for example particularly suitable for labelling an antibody-antigen complex or a double stranded oligo- or polynucleotide without disturbing the complex. This aspect of the invention may also be very useful for visualisation of the entity and/or certain chemical or biochemical processes *in vivo* or *in vitro*.

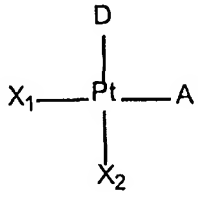
[0020] Examples of preferred platinum compounds are *cis*- or *trans*-platinum compounds of the formula [Pt(II)(X1)(X2)(A)(D)] or a cis-platinum compound of the formula [Pt(II)(X3)(A)(D)].

[0021] Herein, Pt represents platinum (Pt), A and D represent the same or different reactive moieties, respectively involved in the complexation of the platinum compound to a marker and the linking of the platinum compound to the entity. The entities, X1 and X2 represent the same or different inert moieties, and X3 represents an inert moiety that may act as a stabilising bridge, *e.g.* a bidentate ligand.

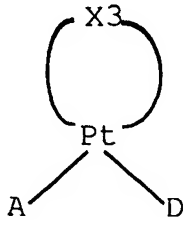
[0022] A structural representation of some examples of such platinum compounds is shown below:



formula 1a



formula 1b



formula 1c

[0023] A platinum(II) compound, for use in a method of the invention can be prepared via any method known in the art. References can for example be found in Reedijk *et al.* (Structure and Bonding, 67, pp. 53-89, 1987). The preparation of some *trans*- platinum compounds is disclosed in EP-A 97201066.4. Further preparation methods can be found in EP-A 96202792.6 and EP-A 95201197.1. Methods described in any of these publications are incorporated herein by reference. In a preferred embodiment of the invention platinum compounds are prepared according to the spacer - *tert* butoxycarbonyl / NHS - label pathway.

[0024] The reactive moieties (A, D) of a platinum compound are preferably good leaving ligands. A platinum compound, wherein A and/or D are independently chosen from the group of Cl^-, NO_3^-, HCO_3^-, CO_3^{2-}, SO_3^{2-}, ZSO_3^-, I^-, Br^-,

F^-, acetate, carboxylate, phosphate, ethylnitrate, oxalate, citrate, a phosphonate, ZO^-, and water has been found to be particularly suitable for use in a method according to the invention. Z is defined herein as a hydrogen moiety or an alkyl or aryl group having from 1 to 10 carbon atoms. Of these ligands, Cl^- and NO_3^- are most preferred.

[0025] Any type of inert moiety may be chosen. Inert as used herein indicates that the moiety remains attached to the platinum compound during the labelling process and thereafter without chemically reacting with an entity. A platinum compound comprising one or two inert moieties chosen from the group of NH_3, NH_2R, NHRR', NRR'R" groups, wherein R, R' and R" preferably represent an alkyl group having from 1 to 6 carbon atoms have been found to be particularly suitable for use in a method of the present invention. H_2NCH_3 is a particularly preferred inert moiety for use in a method according to the invention. An alkyl diamine, wherein the alkylgroup has 2 to 6 carbon atoms is a preferred bidentate inert moiety in a *cis*-platinum compound (*e.g.* X3 in formula 1c). In a particularly preferred embodiment X3 represents ethylene diamine.

[0026] Preferred platinum compounds for use in a method according to the invention include cis[Pt(en)Cl_2], cis[Pt(en)Cl(NO_3)], cis[Pt(en)(NO_3)$_2$], trans[Pt(NH_3)$_2Cl_2$], trans[Pt(NH_3)$_2$Cl(NO_3)], and trans[Pt(NH_3)$_2$(NO_3)$_2$].

[0027] The term labelling is used herein to refer to connecting a marker with an entity, possibly via a platinum linker. A marker as used herein may be any moiety that can be attached to the platinum compound or the entity, and that can be used to detect, monitor or visualise the entity. A marker may be reacted with the platinum compound at any time. Hence, in accordance with the invention it is possible that a platinum linker is first reacted with a marker to obtain a linker-marker complex, which is then reacted with the entity, or that the order is reversed. In a preferred embodiment, the platinum linker is first reacted with the marker.

[0028] Any type of marker may be used as long as it can be attached to the platinum compound. Such a marker may be a radioactive marker, an enzyme , a specific binding pair component such as avidin, streptavidin or biotin, biocytin, iminobiotin, a colloidal dye substance, a phosphorescent marker (*e.g.* an Europium chelate, a platinum porphyrine), a chemiluminescent marker (*e.g.* luminol), a fluorochrome, including a cyanine, a Alexa dye (Molecular Probes), or Bodipy-colourant (Molecular Probes), a rhodamine, dinitrophenol (DNP), carboxyrhodamine, *tert*-butoxycarbonyl, a reducing substance (eosin, erythrosin, *etc.),* a (coloured) latex sol, digoxigenin, a metal (ruthenium), a metal sol or another particulate sol (selenium, carbon and the like), dansyl lysin, a UV dye, a VIS dye, Infra Red Dye, coumarine (*e.g.*amino methyl coumarine), an antibody, protein A, protein G, *etc.*

[0029] Particular preferred are DNP, fluorescein, cyanine-colorants and tetramethylrhodamine, *inter alia* because they can form stable complexes with platinum linked to an entity and they may give rise to excellent limits of detection. These markers can very suitably be used for a technique referred to as multi-colour labelling. Thus several colorants of this kind, optionally having similar chemical structures while having different colours, may be used. Other preferred markers include biotin, avidin, streptavidin and digoxygenin.

[0030] In an embodiment of the invention the marker and/or a reactive site of the entity may be connected to platinum through a spacer. Preferably such a spacer comprises a chain having at least four atoms, and preferably not more than 20 atoms, which chain comprises an electron donating moiety on one end and a moiety for reacting with a marker or an entity on the other end, wherein the chain is attached to platinum through the electron donating moiety. Of course, the spacer(s), the marker, the entity and the platinum linker may be attached to each other in any order. For instance, the spacer(s) may first be attached to the linker followed by reacting the obtained compound with a marker and the entity. It is also possible first to attach the spacer(s) to the marker before the reaction with the linker. The electron donating moiety of the spacer may for example be an amine group or a thiolate anion. Preferably the chain further comprises at least one hetero-atom. Highly preferred spacers are 1,6-diaminohexane and 1,8-diamino-3,6-dioxaoctane. In a preferred embodiment of the invention use is made of 1,6-diaminohexane *tert*-butoxycarbonyl, as an intermediate linker-spacer complex, prior to attaching to a marker and/or entity. It goes without saying that the labelling complex may contain more than one platinum, *e.g.* two platinum atoms, such as for example described in European Patent Appllication 97201066.4.

[0031] One of the reaction parameters that have been found particularly useful to choose such that an entity is differentially labelled in a method according to the invention, is the pH value. The pH as used herein should be interpreted as the pH value of a composition or product according to the invention in water at 20 °C. In case an embodiment of the invention is employed in an environment leading to an altered solvent autoprotolytic constant (pK_w), (*e.g.* presence of organic solvents, altered temperature) a pH mentioned herein should be interpreted based upon the pH range at 20 °C in water.

[0032] In general, the formation of Pt-S adducts is pH independent whereas formation of Pt-N adducts is pH dependent. In a preferred embodiment one or more S-reactive sites are selectively labelled over one or more nitrogen containing sites by making use of the pH.

[0033] As a guideline, in a preferred embodiment, one may choose the pH of the invention at a pH below the lowest pKa of any of an entity's N-reactive sites that should not be labelled, allowing differential labelling of one or more S-reactive sites. As the skilled professional will understand, besides pKa, other factors may play a role, including the influence of the micro-environment in the vicinity of an entity that is to be labelled.

[0034] In a preferred embodiment the S-reactive site or sites are selectively labelled at a neutral or acidic pH. In a more preferred embodiment the S-reactive site or sites are differentially labelled over N-reactive sites at a pH of 5 or less.

[0035] It has also been found possible to label histidine residues distinctively over other N-reactive sites at a pH between about 6 and 8. A residue of a compound as used herein should be interpreted as the compound itself or as part of a larger entity, *e.g.* an amino acid residue in a protein.

[0036] An overview on the formation of Pt-S and Pt-N adducts at various pH values is given in Table 1.

Table 1:

pH dependent formation of Pt-S and Pt-N adducts in proteins			
	pH>10	pH=7	pH<5
S donor(s)	all	all	all
N donor(s)	all	Histidine only	none

[0037] In theory, the formation of a Pt-S adducts is an one step process. A reactive group leaves the platinum compound upon S donating an electron pair to platinum. This process, the direct conversion Pt-X into Pt-S, is believed to be pH independent. On the other hand, N donors require replacement of a reactive group of the platinum compound by oxygen prior to N substitution. First, Pt-X becomes Pt-O and eventual Pt-N. This is a two step scheme in which the first step can be controlled by changing pH. Factors influencing pH of a solution might interfere with Pt-N adduct formation.

[0038] The presence of ions may also be used to control the selectivity of the platinum compound for N-reactive sites. In an embodiment one or more leaving ligands, preferably anionic moieties, are used in the inhibition of labelling a platinum compound to a N-reactive site, in order to enhance differentiated labelling of a S-reactive site. Examples of such leaving ligands include Cl^-, NO_3^-, HCO_3^-, CO_3^{2-}, ZSO_3^-, SO_3^-, I^-, Br^-, F^-, acetate, carboxylate, phosphate, ethylnitrate, oxalate, citrate, a phosphonate, ZO^-, and water. Z is defined herein as a hydrogen moiety or an alkyl or aryl group having from 1 to 10 carbon atoms. Particularly good results have been achieved by using salts comprising an anionic moiety, of which chloride is particularly preferred. The counter ions are preferably alkali cations, alkali earth cations or cations also used to direct the labelling. In a preferred embodiment the total ionic strength of said anionic moieties used in the inhibition of labelling to a N-reactive site is at least 0.1 mol/l. More preferably the total ionic strength is in the range of 0.1 to 0.5 mol/l.

[0039] The presence of metal ions, such as transition metal ions, may also be used for selection of the reactive site to be labelled. In particular such ions have been found suitable to prevent or slow down labelling of an S-reactive site or to make a labelled Pt-S adduct labile, so that effectively one or more N-reactive sites are differentially labelled over said S-reactive site. Within a method according to the invention it is also possible to direct the labelling by making use of geometrical isomers of a platinum compound - *e.g.* a *cis*-platinum compound and a trans-platinum compound, - such that the platinum compound is specifically labelled to either a sulphur containing reactive site or to a nitrogen containing reactive site

[0040] The presence of a bulky inert moiety at the platinum compound may for example be used to prevent labelling at a reactive site of an entity, wherein said reactive site is partially shielded from a platinum compound with a particular stereochemical structure by the structure of the entity. This may for example be the case if the entity has a complex 3D structure, *e.g.* a protein, a conglomerate of molecules, *etc.*

[0041] It is also possible to differentially label an entity according to the invention by first shielding one or more reactive sites that should not be labelled with a shielding moiety and thereafter react a targeted reactive site of the entity with the platinum compound to which also a marker is attached.

[0042] Shielding as used herein is to be interpreted as deactivation of the affinity of a reactive site for a marker, by reaction of the reactive site with a moiety that prevents attachment of a marker directly to said reactive site or complexation of a marker with platinum linked with the reactive site. Preferably the shielding moiety is present in excess over the number of reactive sites that are to be shielded. The preferred reaction time for the shielding process will depend upon the application, and it will be clear to the skilled professional how to choose the reaction conditions.

[0043] In another preferred embodiment the shielding moiety is selectively removed from the shielded reactive site, after the platinum compound has been reacted such that said platinum compound is differentially linked to said entity.

[0044] In a preferred embodiment one or more S-reactive sites may be shielded, e.g. by a trans-platinum compound under conditions as described above, prior to selectively labelling one or more N-reactive sites of one or more entities. Particularly good results have been achieved with Rhodamine *trans*-Pt (trans[Pt(II)$(NH_3)_2$(NH_2-$(CH_2)_6$-NH-rhodamine) Cl](NO_3)) as the shielding moiety. To improve shielding even further the reaction was performed at a pH chosen between 2 and 5, after which the pH was increased to an alkaline pH for labelling N-reactive sites. Other preferred shielding compounds are cadmium, mercury, or zinc complexes.

[0045] The addition of transition metal ions, such as Cu(II), Zn(II) or a mixture thereof has been found to be particularly suitable to selectively remove a *trans*-platinum compound from an S-reactive site , whilst a labelled N-reactive site of a Pt-adduct substantially remains stable.

[0046] The type of solvent may also be used to differentiate the labelling. In particular the reactivity towards N-reactive sites can vary depending upon the solvent. In particular solvents that may act as a ligand to the platinum compound may decrease the reactivity towards N-reactive sites, and thus such a solvent may favour the labelling of S-reactive sites .

[0047] In addition to the parameters as mentioned above a method according to the invention may further be fine tuned by parameters such as temperature, preferably varied in the range between 0 °C and 120 °C, more preferably in the range between 20 °C and 70 °C; reaction time, commonly in the range between 1 min and 48 hours, preferably in the range between 10 min and 24 hours, more preferably in the range between 25 min and 15 hours; concentration of the reagents, molar ratio of the reagents, overall net charge of the platinum labelling complex, and the like. These parameters may be adjusted depending upon the particular application in any way known in the art. The overall net charge of the platinum labelling complex, for example, affects the specificity of Pt-N adduct formation in histidine at neutral pH. Neutral Pt-complexes, such as fluorescein- and cyanine Pt complexes, form Pt-N adducts whereas positively charged platinum labelling complexes, e.g. rhodamine- and dinitrophenol Pt complexes, do not. Positively charged Pt labelling complexes display differential labelling towards N adducts above the isoelectric point of the peptide, protein, and the like. Apart from allowing the selective labelling of N-reactive sites over S-reactive sites or vice versa, a method according to the present invention also makes it possible to differentiate between distinct N-reactive sites or distinct S-reactive sites, by choosing the correct conditions, such as described herein.

[0048] For example, one or more N-reactive sites of histidine residues may be labelled differentially over one or more other N-reactive sites by linking a platinum compound with a marker, and choosing the reaction conditions such that said platinum compound is differentially linked to a histidine residue of said entity. Such a method can be employed in the presence of S-reactive sites-which may be shielded during the labelling of histidine - but also in the absence thereof.

[0049] Thus an entity, such as a peptide or a protein, can be selectively labelled at one or more histidine residues in a mixture of amino acids or other N-reactive site containing entities. In a preferred embodiment differentially labelling of histidine is accomplished by choosing a pH of about 7 and a Pt labelling complex, with a overall neutral charge.

[0050] The selective labelling of a particular type of S -reactive sites or a particular type of N reactive site offers a solution in several application areas. It may for example be used to screen for a particular type of reactive site in a entity of unknown composition or the presence of a particular entity in a sample. (*e.g.* the presence of histidine in an amino acid mixture). Thus in a repeated differential labelling process, several entities can one after another be labelled with a different marker, which may be useful for screening of several components without requiring separation of a sample, *e.g.* by chromatography, electrophoresis and/or mass spectrometry.

[0051] It may also add further specificity towards the labelling in order to avoid labelling at an undesired reactive site *(e.g.* at a functional part of an entity).

[0052] Furthermore discrimination between distinct N-reactive site or distinct S reactive sites, allows the creation of an entity with a multitude of different markers.

[0053] With a method according to the invention one or more labelled entities can be prepared. The invention also relates to such entities, differentially linked with a platinum compound at a N-reactive site or a S- reactive site. The invention further relates to a labelled entity wherein a marker is attached to the entity via a platinum compound linked to a specific reactive site of the entity.

[0054] In a particular embodiment according to the invention, at least one other reactive entity is differentially or non-differentially labelled, after selective labelling of a first reactive site of an entity or a mixture of entities. Such subsequent labelling may take place with a different marker that is reacted with a platinum compound according to the invention, but it is also possible to use another type of labelling reaction known in the art. For example, after differentially labelling an S-reactive site a subsequent labelling may take place with a label that is reactive towards amines.

[0055] In a preferred embodiment subsequent labelling also involves differential labelling. Thus it is possible to prepare an entity to which different markers are labelled at distinct reactive sites.

[0056] Thus it has been found possible to label an entity or a mixture of entities with several of different markers. Accordingly, the invention relates to entities having two or even a plurality of markers. Labelling with more than one marker can be very useful in various applications. It may for example be used to screen for particular entities in a mixture, without needing an analytical separation, *e.g.* screening for the presence of methionine and histidine in an amino acid mixture. In another embodiment it may be used to monitor a process in which a labelled entity is involved, *e.g.* a process in which an entity is split into several entities, each having a different label or *vice versa*. It goes without saying that the invention is not restricted to qualitative analyses but also includes quantitative analyses of differential labelled entities. In principle, a labelled entity may be subsequently analysed using any liquid based analyte analysis system. In a particularly suitable method according to the invention, comprising the analysis of a labelled entity, the labelled entity is analysed using a high throughput screening liquid based multiple analyte analysis system, *e.g.* a flow

cytometry-system.

[0057] The present invention further relates to a diagnostic kit comprising an entity according to the invention. A diagnostic kit according to the invention preferably comprises one or more preparations selected from the group formed by entities differentially linked to a platinum compound at one or more nitrogen containing reactive sites and/or one or more sulphur containing reactive sites, platinum-linker preparations, buffers, marker preparations, transition metal ion preparations, preparations for adjusting the ionic strength and preparations comprising a shielding moiety.

[0058] Another embodiment of the invention relates to a diagnostic kit, for employing a method according to the invention. Such a kit may for example comprise reaction instructions, one or more platinum compounds for labelling the entity, one or more markers, one or more entities according to the invention, one or more test samples, one or more other reagents, one or more test tubes or strips and the like.

[0059] The invention will now further be illustrated by the following nonlimiting examples.

Example 1

[0060] Two amino acids (histidine and methionine, 0.1 mmol each) were dissolved in 500 µl deuterated sodium phosphate buffer (50 mM, pD = 7.00) and incubated at room temperature with a slight excess (0.44 mmol) of [Pt(en)(NH_2-NH-Boc)Cl]$(NO)_3$ (=PtN_3-Cl), wherein Boc is a marker ((en)= ethylenediamine, Boc= *tert*-butoxycarbonyl).The reaction process was monitored using high-resolution NMR (Bruker DPX-300) visualising ^1H and ^{195}Pt nuclei. The results are shown in Figures 1 and 2. The data showed almost completion of the reaction for the S-reactive sites (methionine, Figure 1) within 120 min, demonstrated by change in signal from PtN_3-Cl to Pt N_3-S-adduct whereas the reaction between the N-reactive sites and the platinum compound proceeded slow (Figure 2). After 24 hours only a quarter of the histidine molecules had been labelled.

Example 2

[0061] Bovine serum albumin (BSA) was dissolved in 0.5 x PBS (phosphate buffered saline, pH = 7.4) to a 1 mg/ml solution. To 1 ml sample of the BSA solution, 0.5 mg Rhodamine *cis*-Pt (cis[Pt(II)(en)(NH_2-$(CH_2)_6$-NH-rhodamine)Cl](NO_3)) was added. To another 1 ml sample of BSA solution, 0.5 mg Rhodamine *trans*-Pt (trans[Pt(II)$(NH_3)_2$(NH_2-$(CH_2)_6$-NH-rhodamine)-Cl](NO_3)) was added. Both samples were allowed to react for 16 hrs at 37 °C. Thereafter unbound fluorophores (unbound Rhodamine and unbound Rhodamine-Pt compound) were removed by gel filtration (10 ml Sephadex G50 column, 10 cm length, 1 cm diameter) using 1x PBS as an eluent. Next, the ratios of bound fluorophore per protein (F/P ratio) were determined using the following formula:

$$\text{F/P ratio} = \frac{112.4 \times A521}{95.0 \times [BSA]}$$

wherein A521 (absorbance at 521 nm) was determined using a Ultrospec 4000 spectrophotometer (APB), and [BSA] (protein concentration in µg/µl was determined with BCA reagent (BCA protein assay kit nr. 23225, Pierce)

[0062] Platinum compound to protein ratios (Pt/P ratio) was determined using the following formula:

$$\text{Pt/P ratio} = \frac{68{,}000 \times [Pt]}{195.0 \times [BSA]}$$

wherein [Pt] (platinum concentration in µg/l was determined by atomic absorption spectroscopy. Briefly, the extend of platinum-protein binding was determined by a Perkin Elmer Atomic Absorption Spectrometer 3100 set to a slitband of 0.70 nm to monitor the Pt line at 265.9 nm. The linear range for quantification was 100-1500 ng/mL. Deuterium background correction was used throughout analysis and the sample volume was between 0.020-0.060mL. Furnace parameters were: drying 120°C/90 sec., ashing 1300°C/60 sec., flushing 20°C/15 sec. and atomization at 2650°C/5 sec. Argon gas was used to purge the furnace.

[0063] The results were as follows:

Platinum compound	F/P ratio	Pt/P ratio
Cis	4.1	4.0
Trans	0.9	3.6

[0064] BSA is rich in methionine and cystein residues (S-reactive sites), at the above conditions reaction to N-reactive sites is slow. The Pt/P ratio shows that both the *cis* and the *trans*-Platinum compound successfully react with the

protein. The F/P ratio shows however that under the conditions of this experiment only the marker (rhodamine) is released from the *trans*-platinum compound, while the *cis*-platinum compound remains bound to the protein . This illustrates that a *trans*-platinum compound may be used to shield a reactive site from attachment of a marker to the *trans*-platinum bound reactive site.

Example 3

[0065] Bovine serum albumin (BSA, Sigma; A-9647), Avidin-D (Vector; A-2000) and Goat IgG anti-mouse IgG (total IgG fraction; Dept. Nephrology, Leiden University Medical Centre) were used to be labelled with biotin-Pt (cis[Pt(II)(en)(NH_2-$(CH_2)_2$-CO-$(CH_2)_2$-CO-$(CH_2)_2$-NH-biotin)CL](NO_3)) (KREATECH, ULK001), DNP-Pt (cis[Pt(II)(en)(NH_2-$(CH_2)_6$-NH-DNP)CL](NO_3)) (KREATECH, ULK003), Rhodamine-Pt (cis[Pt(II)(en)($NH_2(CH_2)_6$-NH-rhodamine)CL](NO_3)) (KREATECH, ULK101) and dGreen-Pt (cis[Pt(II)(en)(NH_2-$(CH_2)_6$-NH-dGreen)CL](NO_3)) (KREATECH, ULK301).

[0066] For each labelling of BSA and IgG, 250 µg protein in 250 µl PBS was mixed with 250 µl water containing 125 µg labelling reagent (protein to label ratio = 1:0.5). When needed the volume was adjusted to 0.5 ml with distilled water. The reaction was allowed to proceed for 16 hrs at 37°C. Unbound labelling reagents were removed by gel filtration (SephadexG25, PD10; APB) with TBS/0.05% Tween 20 as eluent. DNP-Pt labelling of avidin-D was chosen to optimise labelling of proteins with none or non-accessible cysteine and methionine amino acids. Avidin-D was labelled at different protein:label ratios and at fixed ratios in 75 mM and 500 mM Na-phosphate-, TrisHCl- or Nacarbonate buffers with pH varying from 7 to 10. Protein concentrations during labelling remained 0.5 mg/ml, whereas label-Pt reagent concentrations varied between 0.25 to 0.75 mg/ml.

[0067] Fluorochrome to protein ratios (F/P ratio) as well as DNP to protein ratios (D/P ratio) were calculated by measuring the absorption at the fluorochrome absorption maximum (DNP: 363 nm, dGreen: 507 nm and rhodamine: 521 nm). A correction factor is introduced which adjusts the measurement for *cis*-platinum contributions at a particular wavelength and protein concentrations are determined using BCA reagent (Pierce; 23225). Calculating protein concentrations at 280 nm is disrupted by A280 nm contributions of the Pt reagent and can not be used. F/P-ratio formulas were then extracted using UV/VIS spectroscopy and Platinum flameless atomic absorbance spectroscopy (Pt-FAAS). Pt-FAAS was used to determine the number of protein-bound platinum compounds, which provided an accurate measurement of bound fluorochromes or DNP-molecules. The formulas used to calculate F/P and D/P-ratios are listed in Table 2.

[0068] Table 3 shows that BSA and IgG contain more platinum bound fluorochromes compared to avidin-D. In case of Rhodamine-Pt: BSA contains 1 fluorochrome/16.6 kD, IgG has 1 fluorochrome/19.5 kD and avidin 1 fluorochrome/82.5 kD. Furthermore, DNP-Pt and Rhodamine-Pt have comparable reactivity and both are more reactive than dGreen-Pt.

[0069] Experiments performed to increase D/P-ratios for avidin labelling are also listed in Table 3. It is shown that increase in pH of the labelling solution from pH 7 to pH 10 hardly increases the D/P-ratio at low salt conditions. A significant increase is found when the same experiment is performed at high salt conditions, however, a maximum D/P-ratio of 2 was found that could not be raised by varying salt or pH conditions. Increase of the label-Pt concentration during labelling was found to increase D/P-ratios further.

Example 4

[0070] Normal goat serum and serum of a goat immunised with mouse IgG, were labelled with DNP-Pt (cis[Pt(II)(en)(NH_2-$(CH_2)_6$-NH-DNP)CL](NO_3)) at a total protein to DNP-Pt ratio of 2:1 (w/w) for 16 hrs at 37 °C. Mouse IgG was immobilised on a micro titre plate in a dilution series of coating concentrations of 0, 0.1, 0.3, 1, 3, 10, 30, 100, 300 and 1000 ng/ml per well. After this coating step the plates were rinsed with PBS-0.05% Tween 20 for three successive times and finally post-coated with 125 µl PBS/2% casein/3% BSA for 30 minutes at 37 °C.

[0071] Next serum was diluted in maleic acid buffer (Roche Diagnostics) to a solution with a protein concentration of 0.5 ng/µl. Next 100 µl of labelled serum was added to the immobilised mouse IgG and was allowed to react for 60 min at 37 °C. The micro titre plate was washed with 1xPBS-0.05% Tween 20 followed by an 1 hour incubation at 37°C with an HRP labelled anti-DNP antibody (#NEN 7-1-99) diluted in maleic buffer. Unbound anti DNP-HRP was removed by 3 washes with 1xPBS-0.05 Tween 20, 1min. each. Next, 100 µl TMB substrate, diluted in a citrate-phosphate buffer pH 5.3, was added to the wells and allowed to react in the dark for 30 min at room temperature (20-22°C). To stop the reaction 100 µl of 1N H_2SO_4 was added. Absorption at 450 nm was determined as a measure for the anti Mouse IgG - labelled according to the invention - bound to Mouse IgG. The results are shown in Figure 3. In contrast to the non-immunised goat serum the experiment with the immunised goat serum showed a signal of bound anti DNP, indicating that anti-mouse IgG has specifically bound to mouse IgG.

[0072] This experiment was repeated with biotin as the marker instead of DNP and anti-biotin instead of anti-DNP.

Similar results were observed.

Example 5

[0073] Micro titre plates (MB, 762070, Griener) were coated with either Rabbit anti-humane IgG (DAKO, A0424), Rabbit anti-humane IgA (DAKO, A0092), Rabbit anti-humane IgM (DAKO, A0426), Rabbit anti-humane IgD (DAKO, A0093), or Rabbit anti-humane IgE (DAKO, A0094). Each antibody was dissolved in 1xPB at a concentration of 10 µg/ml. The micro titre plates were coated with 100 µl overnight at room temperature. Next, the plates were rinsed with rinsing buffer (0.15 M NaCl, 4.9 mM $Na_2HPO_4.2H_2O$, 1.2 mM KH_2PO_4, 0.05% Tween 80, 0.005% thimerasol) and post coated with 150 µl 1xPBS, 2% casein, 3% BSA (30 min at 37 °C). Untreated whole human serum, at various dilution rates ranging from 1:250 up to $1:9.10^5$ (in serum dilution buffer: 0.1 M Tris pH 7, 0.15 M NaCl, 1% BSA, 2% casein, 0.05% Tween 80, 0.025% thimerasol), was added (100 µl) to the anti-humane IgG and anti-humane IgA coated plates and incubated for one hour at 37 °C. The wells were rinsed thoroughly and the detection limit established by using anti-humane IgG-HRP (DAKO, P-214 / stock solution: 1:20 dilution in Stabilzyme Select (Surmodics), finally 1:100 diluted in serum dilution buffer) and anti-humane IgA-HRP (DAKO, P-216 / 1:35 dilution in Stabilzyme Select (Surmodics), finally 1:100 diluted in serum dilution buffer) conjugates and TMB substrate according to standard procedures.

[0074] The same untreated whole humane serum sample was labelled by adding DNP-Pt (cis[Pt(II)(en)(NH_2-$(CH_2)_6$-NH-DNP)CL](NO_3)) in a total protein to DNP-Pt ratio of 4:1 (w/w) and allowing the mixture to react overnight at room temperature. Next, the sample was diluted, added to the plates (100 µl/well), and incubated as above. Detection limit was determined by using anti-DNP-HRP conjugate (#NEN 7-1-99, 1:1000 dilution in serum dilution buffer; 100 µl/well; 1 hour at 37 °C) and TMB substrate (30 min. at room temperature).

[0075] The results were as follows:

Entity	Classical sandwich ELISA	DNP-Pt format
IgG	$1:3.10^5$	$1:2.10^5$
IgA	$1: 8.10^4$	$1:4.10^4$
IgM	n.a.	$1:2.10^4$
IgD	n.a.	$1:2.10^3$
IgE	n.a.	$1:2.10^3$

[0076] All subclasses were shown to maintain their antigen binding capacity.

Example 6

[0077] The effect of ammonium sulphate was evaluated. First proteins were precipitated with either 50, 100, 200 or 400 µl of a saturated $(NH_4)_2SO_4$ solution (30 min on ice - 30 min room temperature - centrifugation). The supernatant was separated from the precipitate. The precipitates were dissolved to a 0.5 mg/ml concentration in 0.5 x PBS (without dialysis). The protein concentration was determined with BCA reagens (Pierce, see above). Next, the re-dissolved precipitate was labelled with DNP-Pt (cis[Pt(II)(en)(NH_2-$(CH_2)_6$-NH-DNP)CL](NO_3)) at a 4:1 ratio (w/w) for 4 hrs at 50 °C. The results are shown in Figure 4.

[0078] Also, the supernatants, transferred to new tubes, were labelled with DNP-Pt. To 0.5 mg protein (in the supernatant) 0.125 of DNP-Pt (cis[Pt(II)(en)(NH_2-$(CH_2)_6$-NH-DNP)CL](NO_3)) was added. The mixture was allowed to react for 4 hrs at 50 °C. The results are shown in Figure 5.

[0079] The results demonstrate that a method according to the invention can be used to label either a entity that has been precipitated in ammonium sulphate or an entity that is dissolved in an ammonium sulphate solution without need for remove any excess ammonium sulphate. The latter is not possible with standard labelling moieties, *e.g.* HNS-esters.

Example 7

[0080] In this example differential labelling is demonstrated by making use of fluorescence resonance energy transfer (FRET). The bio-organic molecule of choice is microperoxidase. Microperoxidase mp-11 (Sigma M6765) consists of 11 amino acids with two N reactive sites (lysine and histidine) and two S-reactive sites (cysteine). The full length sequence of mp-11 is: valine-glutamine - lysine (N) - cysteine (S) - alanine - glutamine - cysteine (S)-histidine (N) - threonine - valine - glutamine. Mp-11 was dissolved in 0.5 x PBS (pH 7.2) at a concentration of 1 mg/ml. A aliquot of this solution (0.25 mg) was labelled with Flu-ULS at a 1:0.25 ratio in 0.5 x PBS (final volume 499.5 µl) at 50 °C for 4 hours. The fluorescein labelled mp-11 solution was purified over a PD-10 column (APB, nr. 17-0851-01). Prior to the

purification of the solution the column was rinsed three times with 5 ml 0.5 x PBS. The fluorescein labelled mp-11 solution was analysed on a Ultrospec 4000 spectrophotometer (APB) Subsequent, fluorescein labelled mp-11 was labelled with rhodamine-ULS (ratio 1:0.25). Labelling was allowed to take place overnight at 4 °C. Next, the solution was purified and analysed as described above.

[0081] The results are presented in Figure 6. The data show that mp-11 is labelled with fluorescein (A470 FAM 50) and rhodamine (A510 FAM 50 Rho 4). An elevated rhodamine specific emission was obvious when the double labelled mp-11 was illuminated at 470 nm (this is the excitation wavelength of fluorescein) (A470 FAM 50 Rho 4). After excitation fluorescein transfers sufficient energy to the nearby rhodamine leading to fluorescence of rhodamine at 570 nm without direct excitation of rhodamine at 510 nm, this is FRET.

Example 8

[0082] Bovine serum albumin (BSA) was labelled with *cis* or *trans* rhodamine-Pt at pH 4 or 7. BSA was dissolved in 1 x PBS (phosphate buffered saline, pH = 7.4) at an amount of 3%. Small aliquots of this solution (3.3 µl) were labelled according the following scheme: (a) plus 25 µl rhodamine *cis*-Pt (cis[Pt(II)(en)(NH_2-$(CH_2)_6$-NH-rhodamine)CL](NO_3)) of a 1mg/ml stock solution in 0.075 M NaAC/citrate buffer pH 4 (final volume 1ml); (b) plus 12.5 µl Rhodamine *trans*-Pt (trans[Pt(II)(NH_3)$_2$(NH_2-$(CH_2)_6$-NH-rhodamine)CL](NO_3)) of a 2 mg/ml stock solution in 0.075 M NaAC/citrate buffer pH 4 (final volume 1ml); (c) same as (a) but in 0.5 x PBS pH 7; (d) same as (b) but in 0.5 x PBS pH 7. In all cases the protein to label ratio is 1:0.25. Labelling took place at 50 °C for 4 hours. Thereafter the labelled BSAs were column purified. Visual evaluation of the samples clearly showed no coloured solution in (b) and (d) whereas (a) and (c) were coloured (c stronger then a).

Table 2: Formulas used to calculate fluorochrome to protein and DNP to protein ratios

BSA-DNP	$\frac{3.78 \times A363}{[BSA]}$	**IgG-DNP**	$\frac{11.67 \times A363}{[IgG]}$	**Av-DNP**	$\frac{5.5 \times A363}{[Avidin]}$
BSA-Rhod	$\frac{1.29 \times A521}{A521}$	**IgG-Rhod**	$\frac{3.63 \times A521}{[IgG]}$	**Av-Rhod**	$\frac{1.95 \times}{[Avidin]}$
BSA-dGreen	$\frac{1.66 \times A507}{A507}$	**IgG-dGreen**	$\frac{3.85 \times A507}{[IgG]}$	**Av-dGreen**	$\frac{2.37 \times}{[Avidin]}$

Table 3:

F/P- and D/P-ratios obtained from labelling experiments				
protein	**label**	**protein:label ratio (µg : µg)**	**labeling buffer**	**F/P ratio or D/P ratio**
BSA	Rhodamine	1 : 0.5	0.25x PBS	4.0
			pH7.4	4.1
			0.5 x PBS	3.6
	DNP	1 : 0.5	1 x PBS	6.1
	dGreen	1 : 0.5	0.5 x PBS	2.4
			0.5 x PBS	
Goat IgG	DNP	1 : 0.5	0.5 x PBS	8.4

Table 3: (continued)

F/P- and D/P-ratios obtained from labelling experiments				
protein	**label**	**protein:label ratio (µg : µg)**	**labeling buffer**	**F/P ratio or D/P ratio**
	Rhodamine	1 : 0.5	0.5 x PBS	7.7
	dGreen	1 : 0.5	0.5 x PBS	3.9
Avidin-D	DNP	1 : 0.5	0.5 x PBS	1.6
	Rhodamine	1 : 0.5	0.5 x PBS	0.8
	dGreen	1 : 0.5	0.5 x PBS	0.3
Avidin-D	DNP	1 : 0.5	TrisHCl	
			500 mM: pH7	0.2
			75 mM; pH7	1.6
			75 mM; pH8	1.4
			75 mM; pH9	1.6
Avidin-D	DNP	1 : 0.5	Na carbonate	
			500 mM: pH8	0.8
			500 mM; pH9	1.2
			500 mM; pH10	1.9
			75 mM; pH8	1.6
			75 mM; pH9	1.5
			75 mM; pH10	2.0
		1 : 1.0	75 mM; pH10	2.4
		1 : 1.25	75 mM; pH10	2.9

Claims

1. A method for differentially labelling one or more entities through a platinum-linker, said entities together comprising one or more sulphur containing reactive sites and one or more nitrogen containing reactive sites, wherein a complex of a platinum compound and a marker is formed, and wherein said platinum compound is reacted with said one or more entities such that substantially only sulphur containing reactive sites or substantially only nitrogen containing reactive sites are linked to said platinum compound.

2. A method according to claim 1 wherein the pH is used to discriminate between labelling of sulphur containing reactive sites and nitrogen containing reactive sites.

3. A method according to any of the preceding claims wherein the ionic strength is used to discriminate between labelling of sulphur containing reactive sites and nitrogen containing reactive sites.

4. A method according to claim 3, wherein said ionic strength is realised by one or more salts comprising chloride, Cl^-, NO_3^-, HCO_3^-, CO_3^{2-}, SO_3^-, I^-, Br^-, acetate, carboxylate, phosphate, sulphate, ethylnitrate, oxalate, citrate, a phosphonate or a mixture thereof.

5. A method according to any of the preceding claims wherein the stereochemical structure of platinum compound is used to discriminate between labelling of sulphur containing reactive sites and nitrogen containing reactive sites.

6. A method according to any of the preceding claims wherein said sulphur containing site or sites are first differentially shielded by a shielding moiety and thereafter said platinum compound is differentially linked to the nitrogen containing reactive site.

7. A method according to any of the claims 1-5, wherein said nitrogen containing site or sites are first differentially shielded by a shielding moiety and thereafter said platinum compound is differentially linked to the sulphur containing reactive site.

8. A method according to claim 6 or 7, wherein said shielding moiety is a *trans*-platinum compound.

9. A method to any of the claims 6-8 wherein the shielding moiety is selectively removed from the shielded reactive site, after the platinum compound has been reacted such that said platinum is differentially linked to said entity.

10. A method according to any of the preceding claims wherein one or more transition metal ions are used in a concentration chosen such that said platinum compound is differentially labelled to a nitrogen containing reactive site.

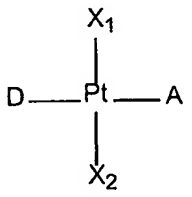
11. A method according to claim 10, wherein said one or more transition metal ions are chosen from the group of Cu (II), Zn(II).

12. A method according to any of the preceding claims, wherein at least one of the nitrogen containing reactive sites is a histidine residue, wherein a complex of a platinum compound and a marker is formed, and wherein said platinum compound is reacted with said one or more entities such that substantially only histidine residues or only non-histidine reactive sites are linked to said platinum compound.

13. A method for labelling one or more entities through a platinum-linker, said entities together comprising one or more histidine residues and one or more other nitrogen containing reactive sites, wherein a complex of a platinum compound and a marker is formed, and wherein said platinum compound is reacted with said one or more entities such that substantially only histidine residues or only non-histidine reactive sites are linked to said platinum compound.

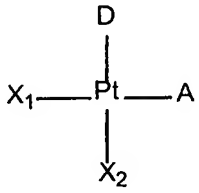
14. A method according to any of the preceding claims wherein nitrogen containing reacting sites and sulphur containing reactive sites are labelled with different markers.

15. A method according to any of the preceding claims wherein, said platinum compound is represented by one of the following formulas:



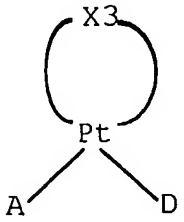
formula 1a

or



formula 1b

or



formula 1c

wherein Pt represents the platinum atom, A and D are independently chosen from the group of reactive moieties formed by Cl^-, NO_3^-, acetate, HCO_3^-, CO_3^{2-}, ZSO_3^-, SO_{32}^-, I^-, Br^-, F^- acetate, carboxylate, phosphate, ethylnitrate, oxalate, citrate, a phosphonate, ZO^-, and water, Z being hydrogen or an alkyl or aryl group having from 1 to 10 carbon atoms;
wherein X1 and X2 are independently chosen from the group of inert moieties formed by NH_3, NH_2R, NHRR', NRR'R" groups, wherein R, R' and R" represent an alkyl group having from 1 to 6 carbon atoms;
and wherein X3 represents a stabilising bridge, such as an alkyl diamine, said alkyl preferably having 2 to 6 carbon atoms.

16. A method according to any of the preceding claims, wherein the marker and/or a reactive site of the entity are connected to the platinum moiety of the platinum compound through a spacer.

17. A method according to any of the preceding claims wherein said marker is a fluorochrome, a phosphorescent marker, chemiluminescent marker, a specific binding pair component, a UV or visual light absorbing marker ,a radioactive marker, a colloidal dye substance , a reducing substance, a particulate sol or a metal.

18. A method according to claim 17 wherein said marker is dinitrophenol (DNP) tetramethylrhodamine, digoxygenin, a cyanine-colorant, biotin, avidin or streptavidin.

19. A method according to any of the preceding claims wherein said entity comprises a primary amine, a secondary amine, a tertiary amine, an aromatic amine, a thiol, a thioether, a sulfide, a disulfide, a thioamide, a thion, an amide, an imide, an imine, an iminoether, or an azide.

20. A method according to any of the preceding claims wherein one or more entities are chosen from the group of amino acids, peptides, oligopeptide, polypeptides, proteins, immunoglobulins, enzyms, synzyms, phospholipides, glycoproteins, nucleic acids, nucleosides, nucleotides, oligonucleotides, polynucleotides, peptide nucleic acids, peptide nucleic acid oligomers, peptide nucleic acid polymers, amines, aminoglycosides.

21. A method according to claim 20 wherein one or more entities are chosen from the group of immunoglobulins, enzymes, antibody-antigen complexes.

22. A method according to any of the preceding claims, wherein the labelled entity is subsequently analysed using a liquid based analyte analysis system.

23. An entity differentially linked to a platinum compound at one or more nitrogen containing reactive sites and/or one or more sulphur containing reactive.

24. An entity according to claim 23 further comprising a marker attached to said platinum compound.

25. An entity labelled with different markers at respectively nitrogen containing reactive sites and sulphur containing reactive sites.

26. A diagnostic kit comprising one or more preparations selected from the group formed by entities differentially linked to a platinum compound at one or more nitrogen containing reactive sites and/or one or more sulphur containing reactive sites, platinum-linker preparations, buffers, marker preparations, transition metal ion preparations, preparations for adjusting the ionic strength and preparations comprising a shielding moiety.

27. A diagnostic kit for employing a method according to any of the claims 1-22.

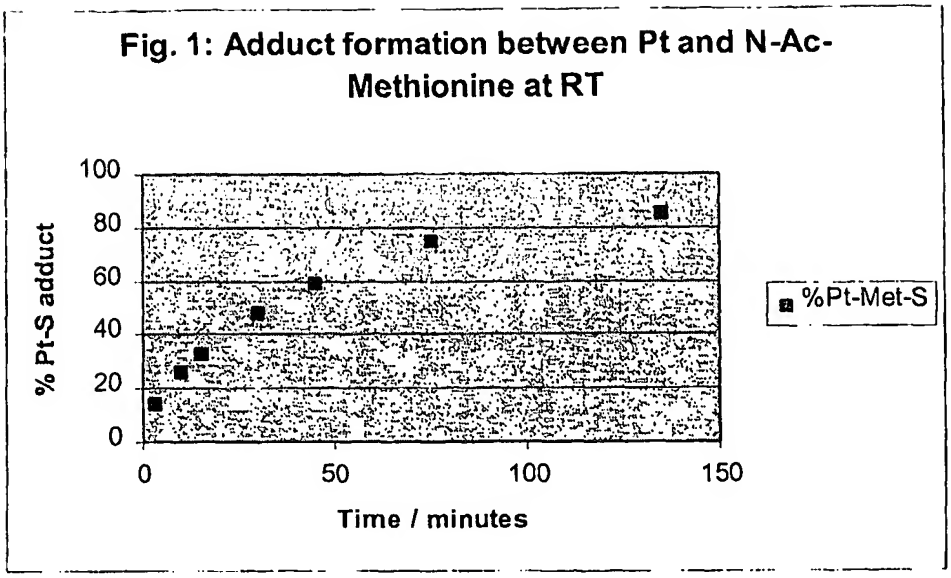


Fig. 1: Adduct formation between Pt and N-Ac-Methionine at RT
% Pt-S adduct
100
80
60
40
20
0
0
50
100
150
Time / minutes
%Pt-Met-S

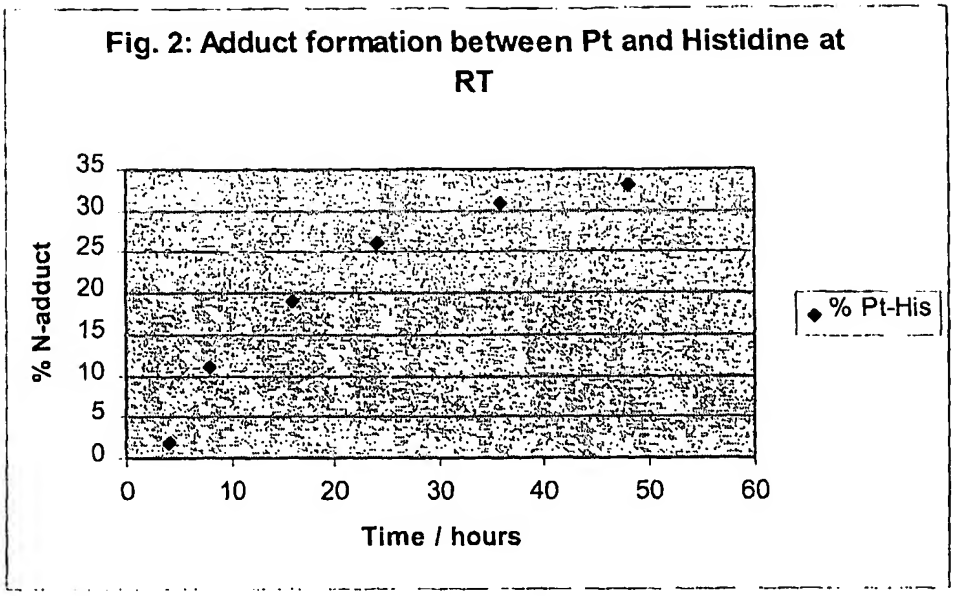
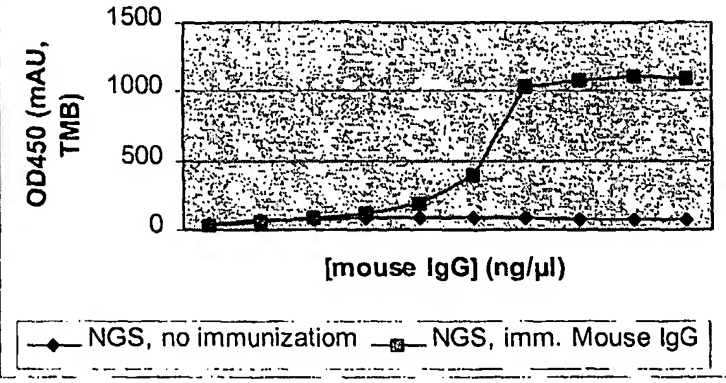


Fig. 2: Adduct formation between Pt and Histidine at RT
% N-adduct
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
Time / hours
% Pt-His

Fig. 3: Labeling of goat sera, non-immunized and immunized with mouse IgG. Mouse IgG coated/DNP-ULS labelled whole serum/anti-DNP-HRP detection



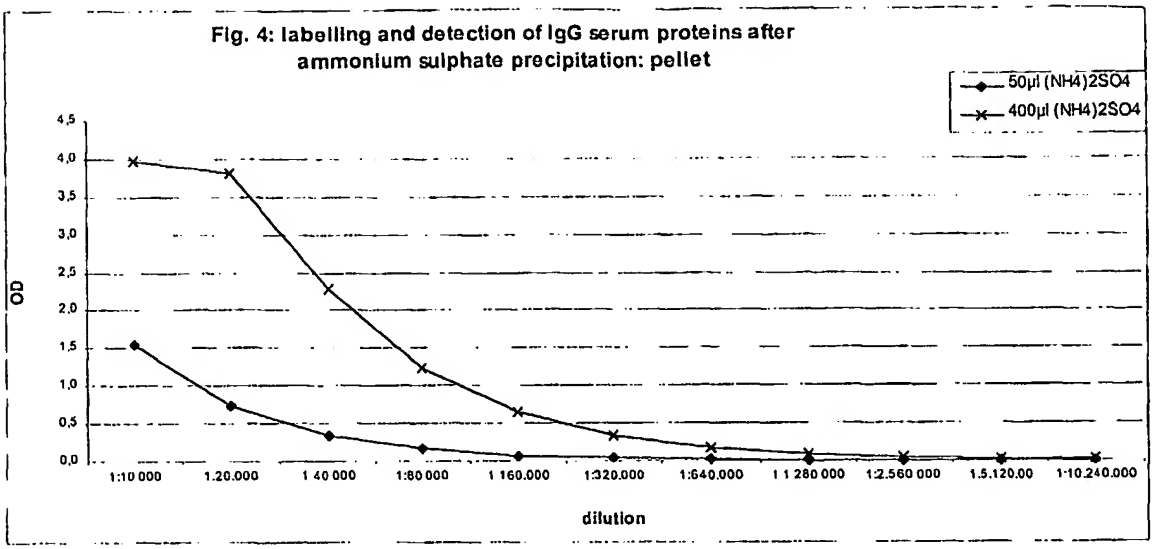


Fig. 4: labelling and detection of IgG serum proteins after ammonium sulphate precipitation: pellet

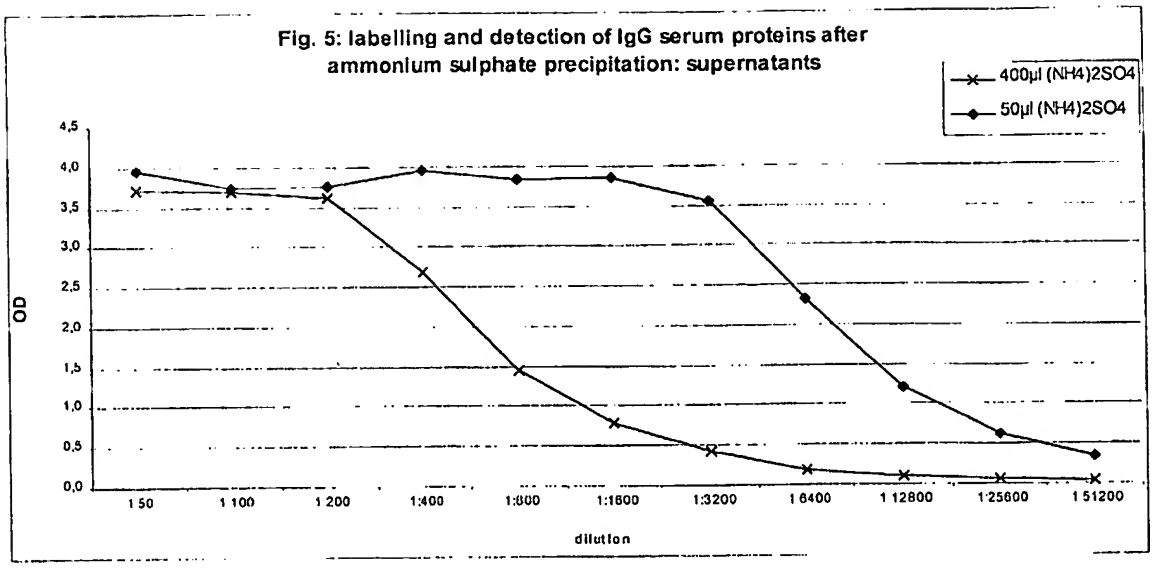
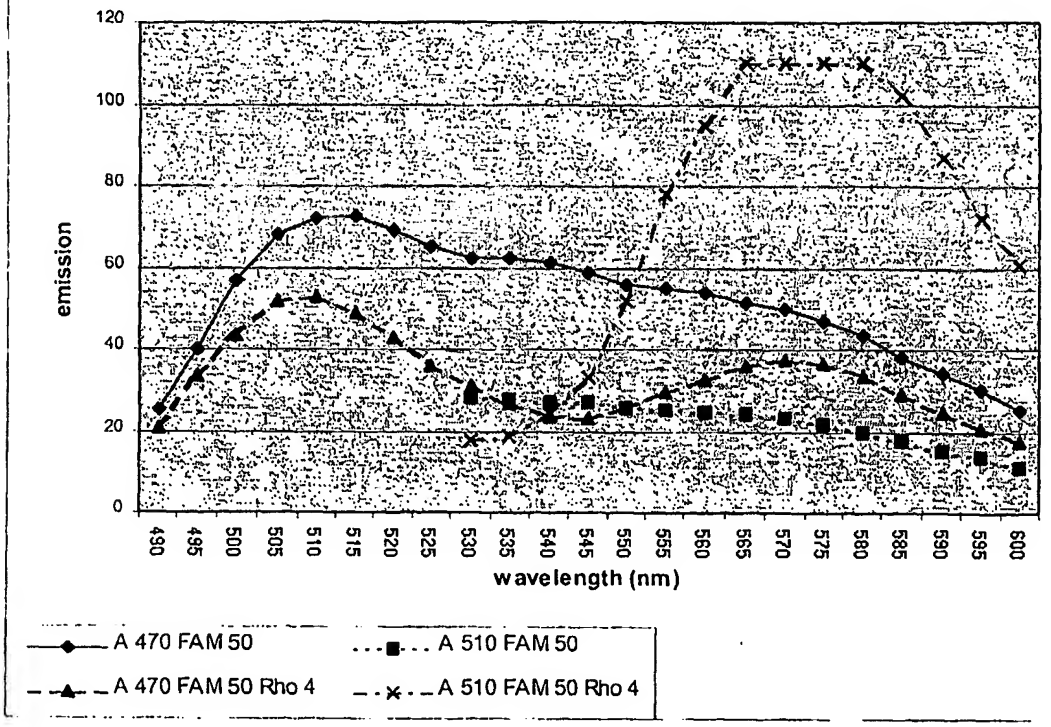


Fig. 5: labelling and detection of IgG serum proteins after ammonium sulphate precipitation: supernatants

Fig. 6: mp-11 double labelling



European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 01 20 2007

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y	WO 96 35696 A (KREATECH BIOTECH BV ;HOUTHOFF HENDRIK JAN (NL); REEDIJK JAN (NL);) 14 November 1996 (1996-11-14) * the whole document * ---	1-27	G01N33/58 G01N33/84
Y	REEDIJK J: "Why does Cisplatin reach Guanine-n7 with competing s-donor ligands available in the cell?" CHEMICAL REVIEWS, vol. 99, no. 9, 8 September 1999 (1999-09-08), pages 2499-2510, XP001064672 * page 2501, column 2 * * page 2508, column 1, line 50 - column 2, line 45 * ---	1-27	
A	WO 98 15564 A (HEETEBRIJ ROBERT JOCHEM ;KREATECH BIOTECH BV (NL); REEDIJK JAN (NL) 16 April 1998 (1998-04-16) * the whole document * ---	1-27	
A	WO 00 27847 A (ARCTIC DIAGNOSTICS OY ;MELTOLA NIKO JARMO JUHANI (FI); SOINI ALEKS) 18 May 2000 (2000-05-18) * the whole document * ---	1-27	TECHNICAL FIELDS SEARCHED (Int.Cl.7) G01N
A	LOWE GORDON ET AL: "The design and synthesis of bis-(4'-Azido-2,2':6',2"-terpyridine platinum(II)) complexes with rigid and extended linkers for studying the topology of DNA by photoaffinity labeling." BIOORGANIC CHEMISTRY, vol. 27, no. 6, December 1999 (1999-12), pages 477-486, XP002195196 ISSN: 0045-2068 * the whole document * --- -/--	1-27	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	10 April 2002	Gunster, M

CATEGORY OF CITED DOCUMENTS

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EUROPEAN SEARCH REPORT

Application Number
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